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Abstract. Amphibian chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), is an emerging infectious disease that widely threatens amphibian biodiversity. However, population-level outcomes following the introduction of the pathogen are highly context dependent and are mediated by a broad suite of biotic and abiotic variables. Here, we examine the effect of the introduction of *Bd* on native island populations of the IUCN red-listed amphibian species *Alytes muletensis*, the Mallorcan midwife toad. We show that the outcome of pathogen introduction is not only dependent on biotic factors, but is also dependent on environmental factors that vary across local scales. Our experimental infections confirm that the genotype of *Bd* occurring on Mallorca is hypovirulent in *A. muletensis* when compared against the lineage found occurring on mainland Iberia. Long-term population data show that *A. muletensis* populations on the island are increasing overall, but trends in highly infected populations are conflicting. We use mathematical models and field data to demonstrate that this divergence in population response to infection can be explained by local environmental differences between infected sites, whereas pathogen genetics, host genetics, and intrinsic epidemiological dynamics driven by fungal load are less likely to be the cause of these differing population trajectories. Our results illustrate the need to take into account the appropriate environmental scale and context when assessing the risk that an emerging pathogen presents to a naïve population or species.

Key words: amphibian; chytridiomycosis; epidemiology; infectious disease; mathematical modeling; state-space time series model; virulence.

Introduction

Emerging infectious diseases caused by fungi pose a major threat to wildlife species (Fisher et al. 2012). This necessitates a focus on host dynamics following disease invasion for the purposes of assessing population viability. The Global Amphibian Assessment has shown that one-third of the world's amphibians are threatened with extinction and that 10% are critically endangered (Stuart et al. 2004). While approximately one-half of amphibian species exposed to habitat loss are not threatened species, species exposed to infectious disease as a threatening process are almost comprehensively at risk (Stuart et al. 2008). Accordingly, EIDs are now recognized as important contributors to the global decline in amphibian species (Bielby et al. 2008). In particular, the disease chytridiomycosis, caused by the aquatic chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has been shown to be instrumental in driving global population declines, local extirpations, and species extinctions (Berger et al. 1998, Longcore et al. 1999, Lips et al. 2006, Fisher et al. 2009*b*). Notwithstanding, host responses to infection by *Bd* are highly variable among species, ranging through the spectrum of complete resistance, through to tolerance, and susceptibility to infection (Daszak et al. 2004, Pounds et al. 2006, Fisher and Garner 2007, Woodhams et al. 2007, Ribas et al. 2009). Individual species can also exhibit highly heterogeneous responses to infection. For example, the spread of *Bd* into mountain yellow-legged frog (*Rana muscosa*) populations in the Sierra Nevada mountains of California has led to rapid, local extirpation of some populations, but persistence of others at low frog densities (Briggs et al. 2010, Vredenburg et al. 2010). This pattern is extended in Europe in common midwife toads (*Alytes obstetricans*) where some populations have experienced high rates of

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mortality and population crashes due to chytridiomycosis, while other comprehensively infected populations appear stable (Bosch et al. 2001, Walker et al. 2010, Tobler et al. 2012).

Species and population-level responses to infection can be affected by immunological and host genetic factors that result in heterogeneity of host responses to *Bd* within and among populations (Woodhams et al. 2007, Ribas et al. 2009, Savage and Zamudio 2011). As well, environmental variables can be key determinants of spatial variation in the severity of epizootics of chytridiomycosis. For example, infection of *A. obstetricans* across the Iberian peninsula was inversely related to exposure to ultraviolet radiation (UVB), and areas with low temperature minima/high altitude significantly increased the risk of mortality (Bosch et al. 2007, Walker et al. 2010, Ortiz-Santaliestra et al. 2011). Similar interactions between temperature and infection have been indicated in Australasian (Kriger and Hero 2007) and Central American ecosystems (Pounds et al. 2006). Experiments have shown how some temperatures aid recovery from chytridiomycosis while others may impair recovery, due to temperature effects on the pathogen, the host, or both (Woodhams et al. 2003, Berger et al. 2004, Ribas et al. 2009). Virulence can also be influenced by parasite genotype (Fisher et al. 2009*a*) and epizootics are associated with a spatially emerging lineage of *Bd* that exhibits heightened virulence (Farrer et al. 2011). Together, these findings show that pathogen-specific as well as host-specific components of risk need to be considered when assessing the overall risk that the introduction of *Bd* poses to a host population or community of amphibians. Nevertheless, some *Bd* epizootics can be explained without evoking host, pathogen, or environmental heterogeneity. Modeling of the *Bd*-load dynamics within populations of *Rana muscosa* indicated that host persistence and extinction outcomes may not have required differences in host susceptibility, pathogen virulence, or environmental conditions. Instead, dynamics observed in wild populations may have resulted solely from density-dependent host–pathogen dynamics, and extinction occurred where a simultaneous build up of high infection intensities in hosts led to host population crash before *Bd* was limited by density-dependent factors (Briggs et al. 2010, Vredenburg et al. 2010).

Our research in a different ecosystem showed that *Alytes muletensis*, the Mallorcan midwife (an IUCN red-listed species), became infected with *Bd* in some populations via the introduction of contaminated individuals. In two of these populations (Torrent des Ferrerets and Coco de sa Bova), infection prevalence consistently measures close to 100%, but to date mortalities have rarely been observed (Walker et al. 2008). *A. muletensis* on Mallorca have been infected by a less virulent (hypovirulent) lineage, *Bd*CAPE, compared to the globalized lineage *Bd*GPL that is associated with amphibian mass mortalities on multiple continents (Farrer et al. 2011). While only a single genotype of *Bd*CAPE has been introduced to Mallorca (Fisher et al. 2009*a*), the two saturated populations show widely differing temporal trends. The Torrent des Ferrerets population has declined and may be close to extirpation, but tadpoles at Coco de sa Bova are increasing in number.

In this study, we investigated the potential causes of these divergent population trajectories. We examined the consequences of emergence of *Bd*CAPE on Mallorca using a *Bd* exposure trial, by analyzing time-series population data to detect if declines are associated with the presence of *Bd*, and by developing mechanistic models of *Bd* and *A. muletensis* population dynamics parameterized to biotic and abiotic aspects of the Mallorcan system. We then extended these models to include temperature-dependence in order to investigate whether these differing population dynamics can be explained by temperature or density-dependence. Finally, we assessed whether the introduction of *Bd*CAPE to Mallorca poses a threat to the continued survival of *A. muletensis* in its natural habitat.

Materials and Methods

Genome resequencing has shown that the Mallorcan genotype of *Bd* is recently descended from a southern African lineage known as *Bd*CAPE, and that this lineage is hypovirulent when compared against the widely globalized lineage *Bd*GPL in common toads (*Bufo bufo*; Farrer et al. 2011). In order to corroborate this finding in *A. muletensis*, we compared the virulence of a representative strain of *Bd*GPL (isolate UKTvB) against the Mallorcan isolate *Bd*CAPE (isolate TF5a1). Seventy-eight captive-bred *A. muletensis* tadpoles were randomly assigned to three treatment groups: (1) *Bd*GPL UK TvB, (2) Mallorca *Bd*CAPE TF5a1, and (3) sham exposure. Animals were individually housed in 0.7-L tubs, received a cumulative exposure of 23 000 zoospores over two weeks, and allowed to complete metamorphosis. The experiment ran for 160 days and all animals were tested for the intensity of infection using a qPCR-based molecular diagnostic assay (Boyle et al. 2004). Kaplan-Meier survival curves and log-rank tests were then used to compare the virulence of the isolates, defined as the severity of the mortality rate inflicted on the individuals exposed to *Bd* compared to that of negative controls (Appendix B). The experiment was performed following full ethical review by Imperial College London, ZSL, and under license by the UK Home Office.

Adult *A. muletensis* are secretive, cryptic, and rarely encountered, while overwintered tadpoles are large, occupy clear, open pools, and are easily enumerated. For this reason, we used estimates of the number of overwintered tadpoles as our index of population size. Mallorcan National Park staff regularly count the number of tadpoles at breeding sites during the summer months, and we collated data for 34 breeding sites on

Mallorca including all four locations where infection had been previously detected (Walker et al. 2008). Because there is observation error (over- and under-counting) in counts of the number of tadpoles (Jung et al. 2002), we used a state-space time series model (de Valpine and Hastings 2002) to estimate population trends, probabilities of population increase or decline (Wade 2000), and to determine whether infection was associated with population dynamics (Appendix A). Specifically, we adapted the model of Tobler et al. (2012) to the Mallorcan midwife toad time series data. Details are given in Appendix A.

We developed a mathematical model following the susceptible-infected (SI) model of Mitchell et al. (2008), but including stochastic effects. The model described the dynamics of the *A. muletensis* population and free-living zoospores of *Bd* within a single water body, with infection-induced mortality occurring in juvenile animals as seen in natural populations and in vivo experiments (Appendix B). As there is currently no experimental work available from which we could derive *Bd* transmission parameters in *A. muletensis*, we parameterized the model using results from our experimental work on *Bd* transmission in *Bufo bufo* (Mitchell et al. 2008, Garner et al. 2009*b*) incorporated with existing field data on *A. muletensis* life history variables (J. Bosch, *unpublished data*). We used the model to estimate whether *A. muletensis*, *Bd*, or both persist 35 years after the introduction of infection and specifically compared the effect of parasite genotype on host population response using the mortality estimates derived from our experiment. Parameter definitions and values are given in Table B1 along with the full set of differential equations in Appendix B.

Our second model investigated the effect of environmental variability on host survival. Recent experimental work in the congener *A. obstetricans* described a relationship between temperature and probability of remaining infected (Geiger et al. 2011) in the form of the following equation:

$$\text{logit}(\text{probability of remaining infected}) = 12.356 - 0.507 \times \text{mean temperature}. \quad (1)$$

To investigate whether this temperature dependent recovery rate could determine the differing population outcomes between Torrent des Ferrerets and Coco de sa Bova, we incorporated the effect of water temperature into the model using measurements collected at both sites via submerged dataloggers for the period August 2008–August 2009. Data show that tadpoles at Coco de sa Bova experience more hours per year above 10°C and with temperatures reaching 33°C. In comparison, tadpoles at Torrent des Ferrerets experience over twice as many hours below 10°C than those at Coco de sa Bova, and are exposed to a maximum temperature of 27.7°C. We extended the basic model (SI) to incorporate a recovered tadpole class (SIR), where infected tadpoles recovering to this class according to a temperature derived rate based on Eq. 1. Tadpoles in the recovered classes were assumed to be immune to further infection and suffered mortality and maturation at rates equal to uninfected individuals. As the immune response to *Bd* in *A. muletensis* is currently not known, sensitivity testing on this assumption was carried out to determine whether tadpoles becoming immune or remaining susceptible post-infection impacted upon the model results. A deterministic version of this model was then combined with the annual temperature profiles collected for Coco de sa Bova and Torrent de Ferrerets. The model was then fitted simultaneously to the population data for the two locations allowing only initial population, population capacity, and time of infection to vary independently between the two populations.

We extended the basic model again to investigate if variation of host density (Briggs et al. 2010) could explain the divergent trajectories of the two heavily infected populations. We modified the initial model to incorporate individual fungal load dynamics, previously shown to be an important determinant of host population dynamics when host density varies and the host is experiencing a *Bd* epizootic (Appendix B; see also Briggs et al. 2010). We used this model to describe, as before, the population dynamics of *A. muletensis* and *Bd* within a single site, and used the same host population structure. We assumed that initial infection occurred during the larval period, and infection-induced mortality occurred at or soon after metamorphosis in all juveniles that developed infections that exceeded an experimentally determined fungal load threshold. The model was used to examine two separate scenarios: a large stable population (equivalent to Torrent des Ferrerets), with the initial population size set to 140 adults and the population limit set to keep population size steady in the absence of disease, and a small, expanding population (equivalent to Coco de sa Bova) with the initial population size set to 25 adults and 100 tadpoles and the population limit set to the same as in the Torrent des Ferrerets scenario. Initial population sizes for the two sites prior to the introduction of *Bd* were estimated from tadpole counts and from population data collected at the estimated time of reintroductions of *A. muletensis* to Coco de sa Bova, which is when *Bd* introduction is presumed to have occurred (Walker et al. 2008; see Appendix B for model structure and outputs).

Results

Exposure to a *Bd*GPL isolate was associated with significantly greater mortality than exposure to either *Bd*CAPE isolated from a Mallorcan midwife or the sham infection ($P = 0.001$; Fig. 1). There was no statistically significant difference in virulence between *Bd*CAPE and the control sham infection, although mortality of midwife toads exposed to the Mallorcan isolate was greater than sham-exposed animals (Table 1). All animals were screened for infection, including

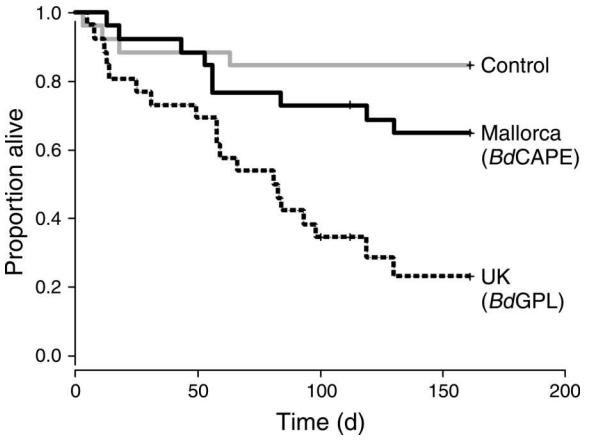

FIG. 1. Changes in survival probability for experimental groups of *Alytes muletensis* tadpoles and metamorphs infected by two different isolates of *Bd* over 160 days: *Bd*GPL, isolate UKTvB; *Bd*CAPE, isolate TF5a1. The sample size is $n = 26$ individuals per experimental group at time 0.

animals that had died. *Bd*GPL proved to be more infectious than *Bd*CAPE, with 83% of animals manifesting detectable infection (intensity of 1994 ± 651 [mean ± SD] genome equivalents) compared to 39% (intensity of 276 ± 195 genome equivalents) of animals infected by *Bd*CAPE; mean intensities of infection found in *Bd*GPL-infected animals were significantly greater than those found in *Bd*CAPE-infected animals (Kruskal-Wallis $P = 0.0004$). No animals in the control treatment were found to be infected.

Mallorcan midwife toads are present at 34 sites in total on the island. Of these, four of the sites are *Bd* positive. Among the uninfected sites, the overall trend in populations is increasing, however, for *Bd*-infected populations, trajectories were inconsistent. The number of tadpoles counted at Torrent des Ferrerets has been decreasing over time, while tadpole counts for Cocó de sa Bova are numerically increasing (Appendix A). *Bd*-free populations had an average growth rate $\hat{\bar{\lambda}} = 0.052$ (95% credible interval 0.007, 0.100) while *Bd*-infected populations had a slightly smaller average growth rate $\hat{\bar{\lambda}} = 0.044$ (95% credible interval −0.382, 0.523). In other words, there is no significant difference in overall population growth between *Bd*-positive and *Bd*-negative populations. The wide 95% credible interval for $\hat{\bar{\lambda}}$ of *Bd*-infected populations reflects the striking among-population variation in the population trajectories of those populations. (Fig. 2, Appendix A).

Our initial SI model revealed that persistence of both *A. muletensis* and *Bd* was highly sensitive to changes in all four *Bd* parameters: zoospore mortality rate, number of zoospores released, transmission parameter, and additional juvenile mortality, as well as parasite genotype. Local extinction of host, of pathogen, and persistence of both were all possible outcomes within the parameter ranges (Fig. 3). However, when post-metamorphic mortality (α) was set to the value observed from laboratory infection of *A. muletensis* with *Bd*GPL ($\alpha = 4.54$), the most likely outcome was the extinction of *Bd* after the initial epizootic followed by a slow host population recovery, but host extinction was still possible over a wide range of parameter values (Appendix B). Increased rates of transmission and increased rates of zoospore release increased the chance of extinction, which was possible within only a few years after the introduction of virulent *Bd* (Fig. 3). Host and pathogen coexistence was unlikely at high values of α, although increasing zoospore life span increased the probability of coexistence at weaker rates of transmission and zoospore release (Fig. 3, Appendix B).

If α was set to the infection-specific death rate for *Bd*CAPE ($\alpha = 0.61$), the most likely outcome was coexistence of host and pathogen (Fig. 3b), but the presence of *Bd* permanently depressed host population size. Host extinction was rare even at the highest rates of transmission and zoospore release (Fig. 3b), and then only after a long, gradual, population decline. Extinction of *Bd* after the initial epizootic was possible over most of the parameter range, and the rate of host population decline before pathogen extinction was dependent on rates of transmission and zoospore release: greater rates of either increased rate of host population decline. Increasing zoospore life span increased the likelihood of both host extinction and host and pathogen coexistence, while decreasing the probability of pathogen extinction. If pathogen-induced mortality was zero ($\alpha = 0$), the most likely outcome was host–*Bd* coexistence. Pathogen extinction occurred only if the initial 20 000 zoospores failed to infect a significant number of tadpoles, and host extinction

TABLE 1. Results of *Alytes muletensis* infection experiment.

Treatment group	No. deaths	*Bd* load†	Cumulative time spent (yr)	Group death rate (yr^{-1})	Isolate-specific death rate (yr^{-1})‡
Control	4/26	0	9.96	0.40	-
Mallorcan CAPE isolate (TF5a1)	9/26	275 (19)	8.93	1.01	0.61 (−0.16–1.37)
UK GPL isolate (TvB)	19/26	1994 (65)	5.63	3.38	2.98 (1.41–4.54)

Notes: Bd load is measured as genomic equivalents using known genome standards. Cumulative time spent is the sum of time in the experiment for all animals in a treatment, group death rates are the number of deaths divided by the cumulative time spent, and isolate-specific death rates are the excess death rates for the two *Bd* treatments over the control treatments.

† Values in parentheses are SE.

‡ Values in parentheses are 95% confidence intervals, which were calculated using the standard error of the difference in rates.

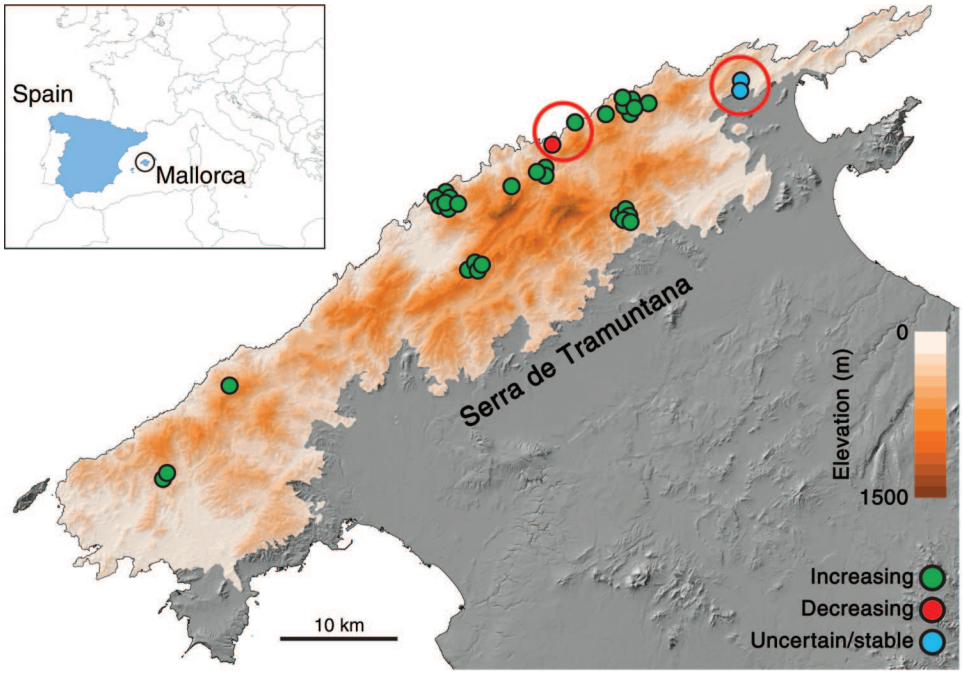

FIG. 2. Map showing the locations of *Alytes muletensis* populations on Mallorca. The population trends are shown for populations with a probability of increase >0.8 (green), a probability of increase >0.5 and <0.8 (blue), and a probability of decline >0.8 (red). Red circles indicate *Bd*-infected populations.

never occurs. Increasing zoospore life span lessened the likelihood *Bd* will not establish.

Readings from the dataloggers show that Coco de sa Bova is a warmer site than Torrent des Ferrerets. Tadpoles at this site are exposed to more hours per year above 10°C and temperatures reaching 33°C, whereas tadpoles at Torrent des Ferrerets are exposed to over twice as many hours below 10°C and a maximum of 27.7°C (Fig. 4). Incorporating site-specific temperature profiles into the model showed that the divergent population size trajectories can be explained by temperature-dependent recovery rates without varying any pathogen or host life history parameters other than those specific to the location (initial population, population capacity, and time of infection; Fig. 4). If the temperature variable was excluded from the model,

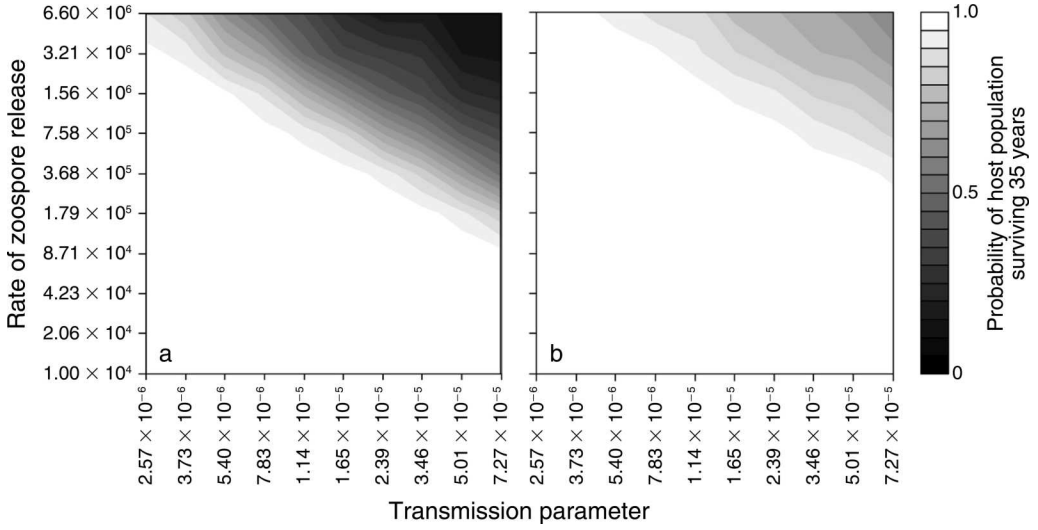

FIG. 3. Dynamics of infected *A. muletensis* populations and *Bd*. Chance of extinction of *A. muletensis* population (from 0 to 1) after 35 years over the full range of ν (rate of infection), ρ (zoospore release rate), and α (mortality) for (a) high virulence (death rate due to zoospores μ_Z), $\mu_Z = 52$ and $\alpha = 4.54$, and (b) weak virulence, $\mu_Z = 52$ and $\alpha = 0.61$.

then the differing trends could not be recovered. Testing the assumption of lack of reinfection produced similar results, indicating the initial result was not dependent on tadpoles becoming immune after clearing infection via elevated temperature.

The effect of host density incorporating fungal load

Altering host density indicated that divergent population trajectories were possible when transmission probability (β) was low and when zoospores remained infectious for longer than a month. Host and parasite coexistence was possible in the small expanding population for several years until the host population density increased sufficiently to trigger a large increase in pathogen density. This increase in pathogen density resulted in a host population crash followed by extinction of the pathogen (Appendix B). Under the same parameter set, the large, stable population suffered a rapid population crash after pathogen introduction. This pattern could not be reproduced using shorter zoospore infectious life spans (less than a month), with the majority of parameters resulting either in extinction of both host and pathogen, a reduction in host population size followed by extinction of the pathogen and recovery of the host population, or immediate extinction of the pathogen. Long-term coexistence of host and pathogen was unlikely in both scenarios. Previous work on *R. muscosa* has suggested that host persistence and extinction outcomes may not require differences in host susceptibility, pathogen virulence or environmental conditions, with density-dependent host–pathogen dynamics explaining variation (Briggs et al. 2010, Vredenburg et al. 2010). Here we find that in *A. muletensis* differing population outcomes can only be explained by host density if zoospore infectious life spans are assumed to be unrealistically long (Woodhams et al. 2008).

Discussion

Despite the introduction of *Bd* onto the island of Mallorca in the 1980s, the pathogen has remained contained to four infected sites out of a potential 34, and none of these infected sites have shown the rapid epizootic declines witnessed in some infected mainland populations of *Alytes obstetricans* (Bosch et al. 2001, Walker et al. 2008). Instead population responses to infection span all possibilities; population size increase, stability, and decline (Appendix A). Our broad conclusion is that the relatively innocuous nature of the host/pathogen dynamic on Mallorca is owed at least in part to the serendipitous introduction of a hypovirulent lineage of *Bd*. Our in vivo assessment confirms that *Bd* lineage-specific patterns of virulence assayed previously in another amphibian species hold true in *A. muletensis* and that the lineage that was introduced onto Mallorca, *Bd*CAPE, exhibits reduced virulence with respect to the global pandemic lineage *Bd*GPL (Fisher et al. 2009*a*, Farrer et al. 2011). Our observation that the prevalence of infection and infectious burden are lower in the *Bd*CAPE vs. *Bd*GPL treatment suggests that *Bd*CAPE is less infectious than *Bd*GPL and possibly less capable of proliferating in *A. muletensis* tadpoles under these conditions. Other experiments involving stronger doses have shown that *A. muletensis* can experience elevated mortality due to this lineage (T. W. J. Garner, *unpublished data*), providing further support that *Bd*CAPE should be considered hypovirulent rather than avirulent (Farrer et al. 2011). Rare field observations of dead, recently metamorphosed, and infected individuals in both of the high prevalence Mallorcan populations suggest that the hypovirulence of this strain can still cause observable mortality in a proportion of naturally infected individuals.

The use of transmission and zoospore release parameters estimated from *B. bufo* data means that results from the SI models should be treated as qualitative outcomes rather than quantitative predictions about the effect of *Bd* on *A. muletensis* populations. However, the lack of field evidence of large-scale mortality and solid evidence of persistent infections at two locations compare favorably with the outputs of the SI model where the experimentally derived *Bd*CAPE mortality rate was incorporated. In agreement with Briggs et al. (2005, 2010) host–pathogen coexistence was unlikely at the high levels of virulence associated with infection by *Bd*GPL. Longer zoospore infectious life spans increased the probability of infection given lower transmission and zoospore release rates, as previously indicated by Mitchell et al. (2008), but this often involved extending zoospore life spans to unrealistic lengths (Woodhams et al. 2008). Higher virulence increased the risk of host extinction in model runs using the more extreme parameter estimates, highlighting the need for strict biosecurity to guard against further introductions of other, more virulent strains of *Bd* to Mallorca. If recombination associated with increased virulence occurs on Mallorca within *Bd*CAPE, as has occurred in other fungal pathogens (Fisher et al. 2012), then there is a concern that more virulent strains may evolve naturally on the island. However, the converse argument equally may apply, and coevolutionary dynamics may lead to a decline in virulence over time. As well, although weaker virulence made host–pathogen coexistence more likely, it did so often at the expense of population size. The costs associated with weakly virulent *Bd* still have the capacity to reduce population numbers to levels where extinction through stochastic effects becomes a strong possibility.

Possible explanations for heterogeneous host population responses to invasion by *Bd* are (1) different *Bd* genotypes, (2) different host genotypes, (3) intrinsic, density-driven dynamics of the pathogen–host system, or (4) environmental variation. Only one genotype of *Bd* has been isolated on Mallorca, and from multiple affected locations, ruling out differing pathogen strains as a driver (Fisher et al. 2009*a*). Despite high levels of

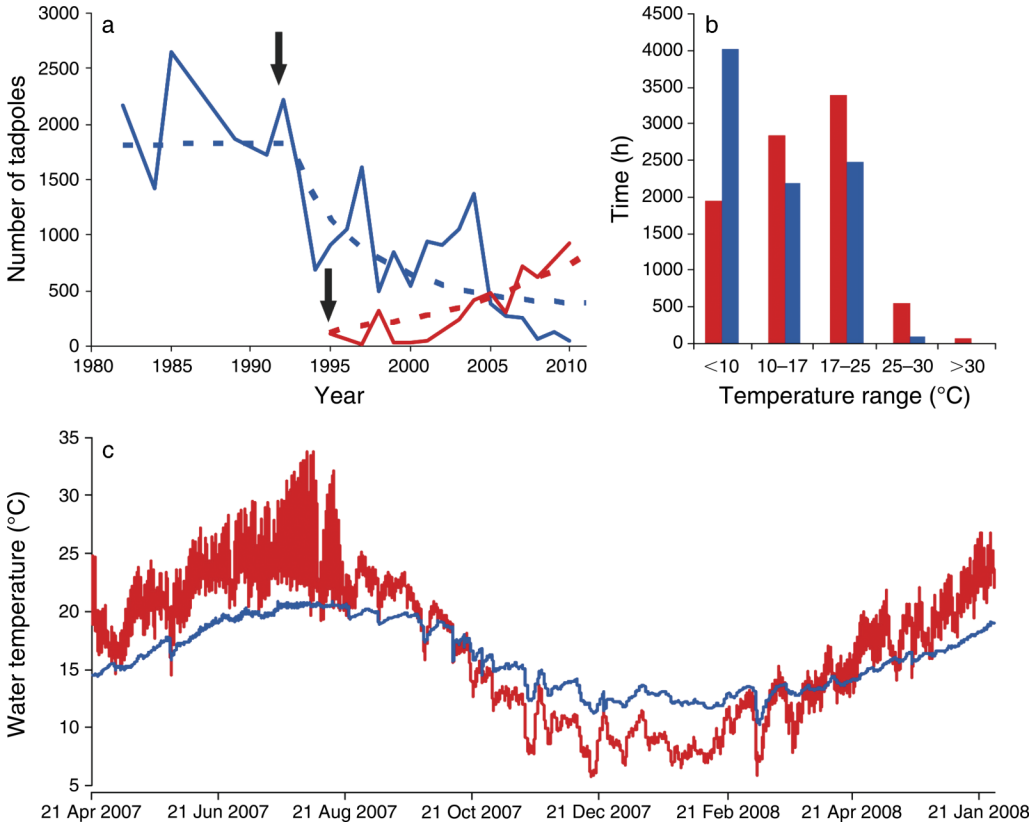

FIG. 4. (a) Solid lines are actual Coco de sa Bova (red) and Torrent des Ferrerets (blue) population data; dashed lines are fitted model results. Parameters are $\nu = 3.1546 \times 10^{-5}$, $\rho = 28\,730$, $\mu_Z = 52$, and $\alpha = 4.54$ for both models. For Coco de sa Bova, the initial adult population is 56 and $K = 3606$, where $K =$ the strength of population regulation. For Torrent des Ferrerets, the initial adult population is 820 and $K = 1581.5$. Arrows indicate time of infection indicated by the model. (b) Number of hours in a year spent within different water temperature ranges for Coco de sa Bova and Torrent des Ferrerets. Below 10°C, more zoospores are produced, and zoospore infectious life span increases. The range 17–25°C represents the optimal growth range of *Bd* in culture. Above 30°C, *Bd* in culture begins to die. (c) Datalogger readings from the two sites.

population structure observed between *A. muletensis* populations (Kraaijeveld-Smit et al. 2005), Coco de sa Bova was founded by individuals bred from Torrent de Ferrerets. Theory predicts that a founder effect should have resulted in declines at Coco de sa Bova, rather than Torrent de Ferrerets, which is contrary to the observed pattern. Prior to infection, Torrent des Ferrerets had a large, established population and Coco de sa Bova a small, newly established one. While density-dependent effects may be able to explain a wide range of differing host population responses (Briggs et al. 2010), they do not appear to be responsible for the differing dynamics in this system. Our mathematical models incorporating fungal loads predicted differing population responses for a small, expanding population of *A. muletensis* vs. a large established one when zoospore infectious life spans is unreasonably long (Woodhams et al. 2008). Furthermore, extended life spans does not result in the long-term host–pathogen coexistence that we observe in the field. In support of this conclusion, 2012 tadpole captures show that the Coco de sa Bova population is now similar in size to Torrent de Ferrerets in the 1990s, without experiencing density-dependent increases in mortality (Torrent de Ferrerets in the 1990s, ~1200; Coco de sa Bova in 2012, ~1300).

Divergent population outcomes across sites instead are most likely due to microscale heterogeneity in a key environmental factor: temperature. Host susceptibility will be a function of two components: the response of *Bd* to temperature and the response of the immune system of the host to temperature. *Bd* growth in culture is optimal between 17° and 25°C, and above 30°C, it begins to die (Piotrowski et al. 2004). Between 7° and 10°C, more zoospores are released, and these stay infectious for a longer time (Woodhams et al. 2008). Low temperatures reduce some components of the amphibian immune systems (Maniero and Carey 1997, Carey et al. 1999, Rojas et al. 2005), and studies show amphibians are less susceptible to *Bd* at higher temperatures (Andre et al. 2008, Ribas et al. 2009). The steep-sided limestone gorge of Torrent des Ferrerets has a more shaded aspect than Coco de sa Bova, which is a shallow pond set out in the open (Fig. 5). This is reflected in their differing annual water temperature profiles, which show that the

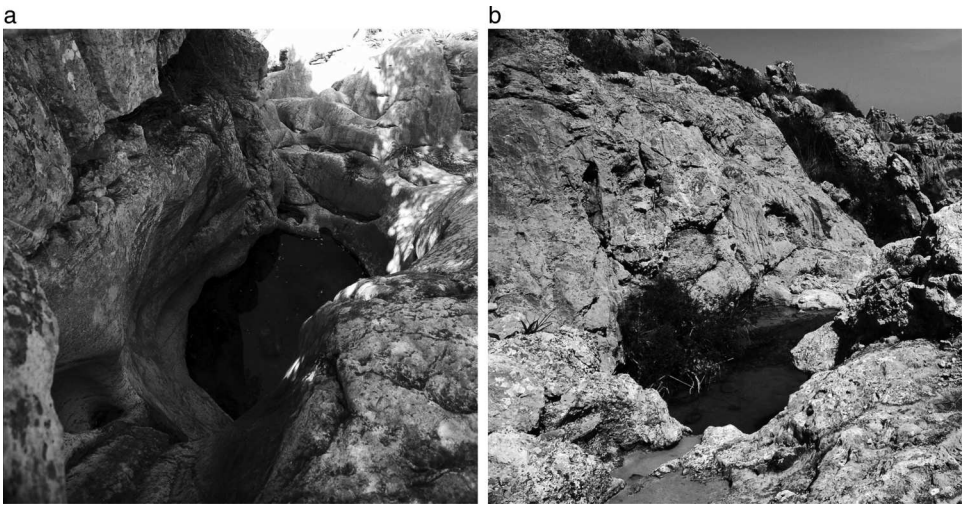

FIG. 5. Study sites: (a) Torrent de Ferrerets; (b) Coco de sa Bova.

water temperature at Coco de sa Bova is consistently warmer, and exhibits more variation, than at Torrent des Ferrerets. While Coco de sa Bova spends most time within the optimal temperature range for *Bd* growth in culture, it also spends more time in the temperature ranges above 25°C. Here, the higher temperatures experienced are likely to regulate the growth rate of *Bd* and may become high enough to directly kill *Bd* (>30°C). The lower temperatures experienced in Torrent des Ferrerets may increase both zoospore infectious life span and zoospore production (factors that this study shows increase the likelihood of population extinctions occurring). Thus it is possible that, while *Bd* is acting as a pathogen and driving population decline within Torrent des Ferrerets, declines are not occurring in Coco de sa Bova due to its higher temperatures. It is interesting to speculate that, in warmer years, the proportion of time that Torrent des Ferrerets is exposed to *Bd*-optimal temperatures will increase, with the likelihood that the dichotomous responses seen between these two populations will be exacerbated. Expanding our model to take into account temperature-dependent recovery using an experimentally determined equation (Geiger et al. 2011) and using the different temperature profiles of the two sites enabled the reproduction of both differing trends. This is consistent with temperature as the factor driving the different host population responses to invasion by *Bd* through its effects on the physiology of *Bd* or the host.

In conclusion, our results show that *Bd* can be considered as both a stable enzootic infection and an epizootic disease within a single ecosystem across small (less than 4 km) spatial scales. However, as *A. muletensis* populations typically occur in colder limestone gorges similar to Torrent des Ferrerets (Moore et al. 2004), and not open pools such as Coco de sa Bova, the spread of *Bd* to other toad populations may precipitate further population declines, indicating a clear and present threat to other *A. muletensis* populations on Mallorca. To quantify this threat, we need to understand in detail the breeding biology of *A. muletensis*, in order to determine the conditions under which *Bd*-induced mortality is additive (and will lead to decline) rather than being compensated by breeding dynamics, leading to stationary or growing populations with enzootic disease (Tobler et al. 2012). This threat suggests that high levels of biosecurity need to be maintained between *A. muletensis* breeding sites in order to prevent the further spread of infection. However, recent findings show that birds may vector the pathogen, lessening our ability to control the spread of *Bd* through habitat management (Garmyn et al. 2012). In light of this finding, pursuing attempts to eliminate infection from Mallorca deserve further attention; despite a lack of success in initial attempts to treat infections in situ with the antifungal itraconazole (Garner et al. 2009*a*, Woodhams et al. 2011), reductions in the intensity of infection lead to cautious hope that further improvements to treatment protocols may work. Our findings emphasize the importance of a good understanding of the context-dependent nature of host–pathogen systems when making conservation decisions, as broad-scale population data alone may have been interpreted as showing no need for concern, especially in the light of new and little-understood emerging infectious diseases (Fisher et al. 2012). More broadly, our work illustrates that a one-size-fits-all approach to assessing the risk that an EID poses to new hosts is naïve, and that the complex interplay of abiotic and biotic factors need to be considered if emergent host-pathogen dynamics are to be correctly forecast.

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Supplemental Material

Appendix A

Time-series and state-space time series models for 34 *Alytes muletensis* uninfected and infected populations (*Ecological Archives* E094-163-A1).

Appendix B

Description, parameterization, and outputs of basic mathematical model 1, and model 2 incorporating fungal load (*Ecological Archives* E094-163-A2).

Supplement

The Berkeley Madonna code to implement the main epidemiological model contained in the study (*Ecological Archives* E094-163-S1).